

# Plant Sterol Esters Lower Plasma Lipids and Most Carotenoids in Mildly Hypercholesterolemic Adults

Joseph T. Judd<sup>a,\*</sup>, David J. Baer<sup>a</sup>, Shirley C. Chen<sup>b</sup>, Beverly A. Clevidence<sup>a</sup>,  
Richard A. Muesing<sup>c</sup>, Matthew Kramer<sup>d</sup>, and Gert W. Meijer<sup>b</sup>

<sup>a</sup>Beltsville Human Nutrition Research Center, ARS, USDA, Beltsville, Maryland 20705,

<sup>b</sup>Unilever Bestfoods NA, Englewood Cliffs, New Jersey 07632,

<sup>c</sup>The George Washington University Lipid Research Clinic, Washington, DC 20037,

and <sup>d</sup>Biometrical Consulting Service, Beltsville Area ARS, Beltsville, Maryland 20705

**ABSTRACT:** The ability of plant sterol esters (PSE) in salad dressing to modify plasma lipids and carotenoids was determined in 26 men and 27 women fed controlled, weight-maintaining, isocaloric diets. Diets contained typical American foods that provided 32% of energy from fat. Dressings contained 8 g (ranch) or 4 g (Italian) of fat per serving. PSE (3.6 g/d) were provided in two servings/d of one of the dressings. Diets with ranch or Italian dressing without and with PSE were fed for 3 wk/diet and crossed over randomly within dressings. Diets were adjusted to similar fat and fatty acid concentrations. Type of salad dressing did not affect plasma lipids, lipoproteins, carotenoids, or fat-soluble vitamins ( $P > 0.05$ ). Switching from a self-selected baseline diet to the control diet resulted in reduction in low density lipoprotein (LDL) cholesterol of 7.9%, a decrease in high density lipoprotein (HDL) cholesterol of 3.1%, and a decrease in triglycerides (TG) of 9.3%. Consumption of 3.6 g of PSE resulted in further decreases in LDL cholesterol (9.7%) and TG (7.3%) but no additional change in HDL cholesterol. Total plasma carotenoids decreased 9.6% with PSE. An automated stepwise procedure was developed to produce candidate mixed models relating plasma carotenoid response to PSE. These models adjusted for preintervention plasma carotenoid levels and effects of diets on blood lipids. There were significant decreases in  $\beta$ -carotene,  $\alpha$ -carotene, and  $\beta$ -cryptoxanthin (females only) not associated with changes in plasma lipids. Plasma carotenoids on all diets remained within normal ranges. We conclude that low-fat foods, such as salad dressings, are effective carriers for PSE.

Paper no. L8820 in *Lipids* 37, 33–42 (January 2002).

Although there is increased public health emphasis on maintenance of body weight and modification of lifestyle factors to reduce the risk of cardiovascular disease (CVD), elevated blood cholesterol remains of great concern and the target of both dietary recommendations (1) and drug treatments to re-

duce CVD risk. The blood cholesterol-reducing effect of plant sterols is well documented (2). However, plant sterols in natural food sources are low, ranging from 0.1 to 0.5 g/d, and therefore may not have substantial impact on cholesterol concentrations (3). Recent studies have demonstrated that plant sterol-enriched margarines lower total and low density lipoprotein (LDL) cholesterol in normocholesterolemic and mildly hypercholesterolemic subjects (4,5). Currently, such table spreads, prepared from vegetable oil and relatively high in fat (32–65%), are the major carriers for plant sterol esters (PSE) available to the public on the open market. PSE is dissolved in the fat phase of these products, termed in food technology as “fat continuous” products; that is, they are “water-in-oil” products in which water is emulsified into a continuous fat phase.

The efficacy of lower fat products as carriers of sterol esters has not been demonstrated in carefully controlled studies of blood lipids and lipoproteins. Examples of fat-based products lower in fat are salad dressings that are similar to those fed in the current study. These are “fat-in-water” products and employ the “water continuous” concept in which the fat is emulsified in a “water continuous” phase. The first objective of the present study was to determine effects of PSE in products in which fat is emulsified in a “water continuous” phase and in which the food carriers’ fat contents are reduced compared with those of margarines, the usual carriers of PSE. Indirectly, we planned to compare the efficacy of these reduced- and low-fat carriers of PSE with blood cholesterol and carotenoid effects reported for higher-fat, “fat continuous” products such as margarines.

Little is known about the effect of baseline blood lipid, fat-soluble vitamin, and carotenoid concentrations on the lipid- and carotenoid-modifying response to PSE consumption. Thus, a second objective of the current study was to examine the relationship of pretrial plasma concentrations of blood lipids and fat-soluble nutrients with the response to PSE consumption. This was achieved by including the appropriate individual pretrial variables as covariates and determining if the slopes of the control vs. PSE diets differed (i.e., if a covariate by treatment interaction was present).

## SUBJECTS AND METHODS

**Study design.** Two types of salad dressing were compared in a parallel arm design. Within each dressing (arm), there was a

\*To whom correspondence should be addressed at Beltsville Human Nutrition Research Center, Building 308, BARC-East, Beltsville, MD 20705.  
E-mail: judd@bhnrc.arsusda.gov

Abbreviations: ANOVA, analysis of variance; Apo A1, apolipoprotein A1; Apo B, apolipoprotein B; BHNRC, Beltsville Human Nutrition Research Center; BIC, Schwartz’s Bayesian information criteria; BMI, body mass index; CVD, cardiovascular disease; HDL, high density lipoprotein; HPLC, high-performance liquid chromatography; LDL, low density lipoprotein; Lp(a), lipoprotein (a); NCEP, National Cholesterol Education Program; PSE, plant sterol esters; SEE, standard error of the estimated or corrected mean; SEM, standard error of the mean; SFA, saturated fatty acid; TC, total cholesterol; TG, triglycerides; USDA, U.S. Department of Agriculture.

randomized crossover between dressing with and without added PSE. The study cohort was divided into two groups balanced for gender, age, body mass index (BMI), and plasma total cholesterol (TC), and the groups were randomly assigned to the salad dressings. Participants consumed each diet for a period of 3 wk followed by blood sampling during the fourth week. Following the final blood sample in the first dietary period, volunteers were switched to the second diet with no washout between periods. There was no crossover between dressing types. The products were fed as part of a carefully controlled, moderately low fat, low saturated fat, weight maintenance diet. Within each dressing, a double-blind protocol was followed.

**Subjects.** The study protocol was approved by the Johns Hopkins University, School of Hygiene and Public Health, Committee on Human Research. Men and women with normal to slightly elevated blood cholesterol concentration were recruited by advertisement in the area of the Beltsville Agricultural Research Center, Beltsville, MD. Minimum eligibility criteria were based on general health, age (25–65 yr), and BMI within 85–120% of gender-specific ideal BMI specified by life insurance reference tables (6). Subjects selected for the study were required to have fasting plasma high density lipoprotein (HDL) cholesterol concentrations greater than 25 mg/dL for men and 35 mg/dL for women and fasting plasma triglyceride (TG) concentrations less than 300 mg/dL. Volunteers who reported taking lipid-lowering drugs, blood pressure medications, or dietary supplements or who had eating habits inconsistent with the study protocol (e.g., those on vegetarian or low-fat diets) were excluded. Volunteers were evaluated by a physician and determined to be in good health with no signs or symptoms of hypertension, hyperlipemia, diabetes, peripheral vascular disease, gout, liver or kidney disease, or endocrine disorders.

Women taking hormones for birth control ( $n = 2$ ) or postmenopausal hormone replacements ( $n = 3$ ) were included in the study with the requirement that they continue the same regimen (type of hormone, schedule, and dose) throughout the dietary intervention. Although smokers and nonsmokers were accepted for the study, only 5 of the 53 participants completing the study smoked. Exercise was not controlled, but subjects were encouraged to maintain their normal exercise patterns (type of exercise, duration, and frequency) throughout the study and were required to record exercise on their daily questionnaire.

**Basal diet and salad dressings.** Ranch and Italian salad dressings with PSE prepared from soybean oil were fed as part of a mixed diet (basal diet) composed of foods commonly eaten in the United States. Control ranch and Italian dressings having no added sterols but which matched the fat and fatty acid concentrations in the test dressings were fed for comparison. The fat level of both the control (–PSE) and the sterol-containing (+PSE) ranch dressing was 27%. The control and sterol-containing Italian dressings had 17 and 13% fat, respectively. The difference in fat content of the two Italian dressings as well as that between the ranch and Italian dress-

ings was balanced in the total diet by adding appropriate amounts of fat and fatty acids in other foods. Two servings, 30 g (2 tablespoons) per serving, of experimental salad dressing were fed each day. Group 1 was fed the basal diet with Italian dressing that provided 4 g of fat per serving while group 2 was fed ranch dressing that provided 8 g of fat per serving. PSE, prepared from soybean sterols, were added to each dressing so that two servings of +PSE dressing provided 3.6 g of PSE, equivalent to 2.2 g/d of free sterol. Salad dressings were prepared by Lipton (Englewood Cliffs, NJ).

**Dietary intervention.** Monday through Friday, all subjects consumed breakfast and dinner at the Beltsville Human Nutrition Research Center (BHNRC) human study facility under the supervision of a dietitian. At breakfast, each subject was provided with a carry-out lunch to be consumed that day. Snack items were included in the daily menu, and subjects were provided the option of consuming the snacks at dinner or later in the evening. One serving of the salad dressing was provided with lunch, and one serving with dinner. Meals for the weekend were packaged for home consumption and provided to the subjects, with written instructions, after dinner on Friday. Weekend menus contained the same type of foods and balance of nutrients as the weekday menus. Unlimited amounts of coffee, tea, and diet sodas were allowed, but all additives (sugar and milk) for coffee and tea were provided with the meals. Only foods provided by the Human Study Facility were allowed to be consumed during the study.

Each weekday morning, subjects were weighed before breakfast when they arrived at the facility. Energy intake was adjusted in 200-kcal increments for women and 400-kcal increments for men to maintain initial body weight. Subjects were fed the same items and the same proportions of each item relative to total dietary energy. Therefore, the relative amounts of nutrients, other than those provided by the salad dressing, were constant for all subjects. Each day, subjects completed a questionnaire detailing beverage intake, factors related to dietary compliance, exercise, medications, and illnesses. The questionnaires were routinely reviewed by a study investigator, and problems were discussed with the subject during the next meal.

**Analysis of diets.** Two composites of the 7-d menu cycle were made at two energy levels. Thus, four weekly diet composites were analyzed for dry matter, crude protein, crude fat, total dietary fiber, and ash (Corning Hazleton, Inc., Madison, WI). Fatty acid compositions of food composites were determined by gas chromatographic separation of fatty acid methyl esters. Carotenoid content of the controlled diets was estimated using data for carotenoids in foods which were compiled at BHNRC (7).

**Blood sample collection and analysis.** Baseline samples were collected on 2 d during the week immediately before initiation of the controlled feeding. Subsequently, samples were collected on two different days during the fourth week of the intervention. The subjects were randomly divided into two groups. One group had samples drawn on Monday and Wednesday and the other on Tuesday and Thursday.

Procedures for collection and processing of fasting blood samples were those described in the protocol for the Lipid Research Clinics Program (8). Plasma was harvested and stored in cryogenic vials at  $-80^{\circ}\text{C}$ . Before storage, the sample to be used for HDL cholesterol determination was precipitated by the sequential precipitation procedure of Gidez *et al.* (9). Supernatants from the HDL precipitation were stored at  $-80^{\circ}\text{C}$  for later analysis of cholesterol. Analyses for TC, TG, HDL, lipoprotein (a) [Lp(a)], and apolipoproteins were performed after the final blood collection. All samples from one subject were included in the same analytical run.

Lipid and lipoprotein analyses (TC, TG, HDL cholesterol, and apolipoproteins) were performed at the Lipid Research Clinic Laboratory, The George Washington University Medical Center, which maintains standardization with the Centers for Disease Control and Prevention, U.S. Department of Health and Human Services. Plasma TC, HDL cholesterol, and TG were determined enzymatically with commercial kits (Sigma Chemical Company, St. Louis, MO) on an Abbott VP analyzer (Abbott Laboratories, Chicago, IL). LDL cholesterol was calculated by the Friedewald equation (10). Plasma apolipoproteins A1 (Apo A1) and B (Apo B) concentrations were determined by rate nephelometry (Beckman ICS Immunochemical Analyzer; Beckman Instruments, Fullerton, CA).

Carotenoids from 0.4 mL of plasma were extracted into organic solvents containing an internal standard (11), concentrated, and analyzed by high-performance liquid chromatography (HPLC) with a Hewlett-Packard (Wilmington, DE) Series 1050 chromatograph with diode array detection. Carotenoids were separated on a reversed-phase  $\text{C}_{18}$  analytical column (Microsorb-MV; Varian Analytical Instruments, Walnut Creek, CA),  $250 \times 4.6$  mm, protected by a  $5\text{-}\mu\text{m}$  Brownlee  $\text{C}_{18}$  guard cartridge,  $30 \times 4.6$  mm, under isocratic conditions (12). The precision and accuracy of the HPLC system were verified using Standard Reference Material 968b (National Institute of Standards and Technology, Gaithersburg, MD).

**Statistical analysis.** There were a large number of independent variables, and potential interactions among them, that we wanted to consider as predictors of response to PSE consumption for each of the blood lipid, lipoprotein, and carotenoid variables. Therefore, we developed an automated stepwise model selection procedure (13), used for each of the dependent variables. Our procedure selected a mixed analysis of variance (ANOVA) model that minimized Schwartz's Bayesian information criteria (BIC) (14), where a smaller BIC corresponds to a better "fitting" model, given all the candidate independent variables. The basic within-individual covariance structure remained unchanged (that of a crossover design) while selecting the independent variables.

Models were tested in a standard stepwise approach (15). All models included an intercept term. Selection started by adding the single best independent variable or interaction term to the model. With that variable in the model, the next best variable was added. This process was repeated until no other variables could be added. After five or more independent variables were in the model, all variables currently in the

model were also tested to see if any could be removed. We used the resulting "final" model as a basis for further model development, usually by eliminating variables that changed BIC only marginally, because the resulting simpler models were easier to interpret and seemed to "fit" about as well, based on BIC.

For all dependent variables, one important predictor was the variable's pretreatment value, which we refer to as baseline. This is because subjects with high posttreatment values were likely to come into the experiment with high pretreatment values, just as those with low posttreatment values came in with low pretreatment values. The baseline value of the dependent variable adjusts posttreatment scores by the variable's initial value, resulting in a more sensitive test of the treatment effects because much of the "noise" due to initial intersubject variation has been statistically removed. Since it was not initially evident to us that there would be a linear relationship between pre- and posttreatment values, we allowed for nonlinearity by including a quadratic effect (the square of the baseline concentration of the dependent variable) as a candidate independent variable. In general, the quadratic effect was not included in the final model, that is, there was a linear relationship between pre- and posttreatment values.

Other candidate independent variables for both blood lipids and carotenoids included characteristics of the individual (age, sex, and BMI), pretreatment plasma concentration of the dependent variable (baseline, baseline squared), design variables (period and sequence in which the diets were administered and carryover effect), and the variables of most interest, treatment and dressing. First-order interaction terms among these variables were also candidate independent variables. In addition, because carotenoids are carried by lipoproteins and concentrations may be dependent on changes in blood lipids (16), blood lipid variables were included as candidate covariates in models for carotenoids (i.e., TG, TC, LDL cholesterol, HDL cholesterol, and the sum of TG + TC).

For the carotenoids, there were 57 candidate independent variables (not including the intercept) for the routine to select from. There were fewer, 49, candidate variables for the blood lipids than for carotenoids, because blood lipid variables, other than the baseline level of the dependent variable, were not allowed into the model. For example, LDL cholesterol was not adjusted using HDL cholesterol, TC, or TG.

## RESULTS

**Subjects.** Twenty-eight men and 28 women completed the screening process and began the controlled feeding. One woman and two men withdrew for personal reasons not related to the study. Thus, 26 men and 27 women completed the feeding phase of the study. Data were analyzed statistically only for subjects who completed both feeding periods. Characteristics of these participants at baseline are presented in Table 1.

**Diets and salad dressings.** The background diets with salad dressing added were planned to be prudent, healthful diets having moderate levels of fat but lower levels of satu-

**TABLE 1**  
**Characteristics of the Participants at Baseline<sup>a</sup>**

	Men ( <i>n</i> = 26)	Women ( <i>n</i> = 27)	All ( <i>n</i> = 53)
Age (yr)	45.8 ± 1.34	48.4 ± 1.68	47.1 ± 1.54
Body mass index (kg/m <sup>2</sup> )	26.7 ± 0.37	25.5 ± 0.73	26.3 ± 0.37
Plasma concentration (mg/dL)			
Total cholesterol	214 ± 5.0	220 ± 5.6	218 ± 3.8
Low density lipoprotein (LDL) cholesterol	141 ± 4.4	139 ± 4.0	140 ± 2.9
High density lipoprotein (HDL) cholesterol	46.3 ± 2.98	56.4 ± 2.65	51.4 ± 2.09
Ratio of total to HDL cholesterol	5.0 ± 0.23	4.2 ± 0.20	4.5 ± 0.16
Triglycerides	135 ± 12.4	123 ± 11.7	129 ± 8.5
Apolipoprotein A1	197 ± 5.9	213 ± 5.2	205 ± 4.1
Apolipoprotein B	92 ± 2.6	97 ± 2.9	95 ± 2.0
Lipoprotein (a)	22 ± 4.3	23 ± 3.6	23 ± 2.8

<sup>a</sup>Sample mean ± SEM.

rated fatty acids (SFA) and cholesterol, as well as higher levels of dietary fiber than reported for the average U.S. diet (17). Diets were calculated using data from the U.S. Department of Agriculture (USDA) nutrient database (18) so that total fat would be approximately 30% of energy and protein 16% of energy. Analyzed macronutrient and fatty acid compositions of the basal diet and diets with salad dressings are presented in Table 2. Analyzed compositions of diets as fed

in the study showed 32.2–32.7% of energy from fat and 15.7–16.2% from protein. The values for dietary fat are slightly lower than those reported for the U.S. diet (17). In order to avoid confounding the effect of dietary fatty acid intake on blood lipids with those of PSE, considerable effort was made to formulate diets that would vary little in the major SFA (myristic, palmitic, and stearic acids), as well as in major mono- and polyunsaturated fatty acids (oleic and linoleic

**TABLE 2**  
**Composition of Diets Containing Ranch or Italian Dressings With and Without Supplementation with Plant Sterol Esters (PSE) Prepared from Soybean Oil**

	Diets with:			
	Ranch dressing		Italian dressing	
	–PSE ( <i>n</i> = 12 men + 14 women)	+PSE	–PSE ( <i>n</i> = 14 men + 13 women)	+PSE
Added sterol esters (g/d)	0	3.6	0	3.6
Free sterol equivalents added (g/d)	0	2.2	0	2.2
Percent of energy <sup>a</sup> from:				
Protein <sup>b</sup>	16.2	15.7	16.5	15.8
Fat <sup>b,c</sup>	32.7	32.2	32.5	32.7
From 2 servings of dressing <sup>d</sup>	5.5	5.5	2.8	2.8
From basal diet <sup>d</sup>	27.2	26.7	29.7	29.9
Saturated fatty acids (SFA) <sup>b,c</sup>	7.9	7.6	7.6	7.4
Myristic acid (14:0)	0.5	0.4	0.4	0.4
Palmitic acid (16:0)	4.5	4.3	4.2	4.5
Stearic acid (18:0)	2.4	2.3	2.3	2.2
Other SFA	0.5	0.6	0.7	0.3
Monounsaturated fatty acids (MUFA) <sup>b,c</sup>	11.2	10.9	11	11
Oleic acid (all 18:1 isomers)	10.7	10.4	10.5	10.7
Other MUFA	0.5	0.5	0.5	0.3
Polyunsaturated fatty acids (PUFA) <sup>b,c</sup>	10.3	9.9	10	9.8
Linoleic acid (18:2)	9.1	8.7	8.8	8.6
Other PUFA	1.2	1.2	1.2	1.2
Dietary cholesterol (g/d/1000 kcal)	88	97	94	92
Dietary fiber (g/d/1000 kcal)	13	12	11	13

<sup>a</sup>Average energy intake for men was 2766 kcal/d; for women, 2163 kcal/d.<sup>b</sup>Diet composites with salad dressing included were analyzed at 2200 and 3200 kcal. Values are the average of the two composites.<sup>c</sup>Ratio of fatty acids, PUFA:MUFA:SFA = 1.3:1.4:1<sup>d</sup>Percentages of energy (en%) from salad dressing fat calculated at 2200 and 3200 kcal were, for ranch, 6.5 en% at 2200 kcal and 4.5 en% at 3200 kcal, and, for Italian, 3.3 en% at 2200 kcal and 2.2 en% at 3200 kcal.

acids). Ratios of polyunsaturated to monounsaturated to saturated fatty acids averaged 1.3:1.4:1 and were very consistent across diets. The diets averaged 12 g of dietary fiber per 1000 kcal. Mean total dietary fiber intake was 33 g/day for men and 26 g/day for women. These values are considerably higher than the average intake reported for the U.S. diet, 17.4 g/d for men and 13.7 g/d for women (17), but were constant across all diets.

**Blood lipids and lipoproteins.** As expected from documented inherent gender differences, women had higher concentrations of HDL cholesterol (+10 mg/dL) and apo A1 (+16 mg/dL) than did men but were not different from men in concentrations of LDL cholesterol and apo B. The ratio of total/HDL cholesterol was higher for men than for women,  $4.96 \pm 0.23$  vs.  $4.12 \pm 0.20$ .

The effects of type of salad dressing, gender, and gender  $\times$  dressing interaction were found to be nonsignificant ( $P > 0.05$ ) in the mixed ANOVA model; thus, data for the type of salad dressing and gender are combined. Blood lipid and lipoprotein changes due to the addition of 3.6 g PSE/d to the controlled diets are shown in Table 3. Inclusion of 3.6 g PSE/d in the controlled diet resulted in highly significant reductions ( $P < 0.005$ ) in TC [6.8% (13.8 mg/dL)], LDL cholesterol [9.8% (12.5 mg/dL)], apo B [3.3% (3.03 mg/dL)], and TG [7.3% (8.5 mg/dL)]. HDL cholesterol was unaffected by including PSE in the controlled diet ( $P = 0.33$ ). As with HDL cholesterol, apo A1 was unaffected by PSE ( $P = 0.56$ ).

The effect of PSE on the ratio of TC to HDL cholesterol depended on the ratio prior to dietary intervention, that is, there was a treatment by baseline interaction. Even so, PSE consumption always resulted in lower ratios. At a baseline ratio of 4.5, the average of the ratio at the start of the study (Table 1), the ratio decreased by 0.36 (7.5%,  $P = 0.0001$ ). At the minimum and maximum baseline ratio, the decrease was 6.9 and 7.7%, respectively. There were no significant interactions of gender or BMI with PSE effect on the ratio.

**Carotenoids.** There were no differences in the effect of PSE consumption on plasma carotenoid concentrations between Italian and ranch dressings. There were no significant interactions of gender with PSE consumption except for  $\beta$ -cryptoxanthin, where there was a significant treatment effect for females, but not for males (Table 4).

All plasma carotenoids decreased to some degree with consumption of PSE (Table 4). This general decrease in carotenoids was reflected by a 9.6% decrease ( $P < 0.0022$ ) in the sum of those carotenoids determined in our analytical procedure, i.e., total carotenoids. Among the major plasma carotenoids,  $\beta$ -carotene and  $\alpha$ -carotene decreased by 12.7 ( $P = 0.0009$ ) and 12.8% ( $P = 0.0226$ ), respectively. Lycopene decreased with consumption of PSE; however, the magnitude of the decrease varied directly with BMI (i.e., there was a significant treatment  $\times$  BMI interaction (Table 4). Estimation of the effect of BMI showed that the decrease in lycopene due to PSE became statistically significant ( $P = 0.05$ ) at BMI between 22 and 23 and ranged from 4.1  $\mu$ g/dL at BMI 22 ( $P = 0.0843$ ) to 10.9  $\mu$ g/dL at BMI 30 ( $P = 0.0001$ ). At the average BMI in this study (Table 1), the decrease in lycopene would approximate 20% of the mean lycopene concentration for the control (–PSE) treatment. Decreases in the lutein–zeaxanthin carotenoid fraction, anhydrolutein,  $\alpha$ -cryptoxanthin, and  $\beta$ -cryptoxanthin (for males only) due to PSE consumption were not statistically significant ( $P > 0.05$ ).

**Fat-soluble vitamins.** Modeling of  $\alpha$ - and  $\gamma$ -tocopherol and retinol by the same procedure and for the same covariates used for carotenoids showed no significant effects of dressing or treatment and no significant interactions of baseline concentration of the fat-soluble vitamin, gender, or BMI with dressing or treatment. The estimated means and standard errors of the estimated means (SEE) are shown in Table 4.

**Relationships of baseline subject characteristics with blood lipid and lipoprotein responses to PSE.** In Table 5 we present coefficients for independent variables entering into the model with probability values of  $\leq 0.05$  for the lipid and lipoprotein dependent variables. The coefficient for the intercept is included for completeness. There were no significant effects or interactions of type of dressing and treatment in the final model, indicating that the response to PSE consumption (treatment) was the same for both Italian and ranch dressings (Table 5). As expected, baseline concentration was a significant predictor of final concentration for all blood lipids and lipoproteins. However, there was no significant interaction of the baseline concentration with PSE (treatment effects), so the magnitude of change in the plasma lipid was the same over all baseline levels; that is, the effect of treatment on a

**TABLE 3**  
Plasma Lipid, Lipoprotein, and Apolipoprotein Concentrations of Men and Women Consuming 3.6 g PSE/d<sup>a</sup>

Plasma concentration (mg/dL) <sup>b</sup>	(LSMean $\pm$ SEE) <sup>c</sup>			<i>t</i> -test ( <i>P</i> -value)
	Without added PSE	With 3.6 g PSE/d	Difference	
Total cholesterol	202 $\pm$ 1.7	187 $\pm$ 1.7	–13.8 $\pm$ 1.6	<0.0001
LDL cholesterol	129 $\pm$ 1.5	116 $\pm$ 1.5	–12.5 $\pm$ 1.5	<0.0001
HDL cholesterol	50 $\pm$ 0.6	50 $\pm$ 0.6	0.4 $\pm$ 0.44	0.3353
Triglycerides	117 $\pm$ 3.7	109 $\pm$ 3.7	–8.5 $\pm$ 2.9	0.0047
Apolipoprotein A1	199 $\pm$ 2	198 $\pm$ 2	–0.9 $\pm$ 1.6	0.5996
Apolipoprotein B	93 $\pm$ 0.8	90 $\pm$ 0.8	–3.0 $\pm$ 0.7	<0.0001

<sup>a</sup>See Tables 1 and 2 for abbreviations.

<sup>b</sup>Estimated mean and standard error of the estimate from a mixed model analysis of variance that adjusted for baseline subject characteristics, type of dressing (ranch or Italian), period, and carryover;  $n = 53$  (26 men, 27 women).

**TABLE 4**  
**Plasma Carotenoid and Fat-Soluble Vitamin Concentrations of Men and Women Consuming 3.6 g PSE/d<sup>a</sup>**

Plasma concentration (µg/dL)	(LSMean ± SEE) <sup>b</sup>			<i>t</i> -test ( <i>P</i> -value)
	Without added PSE	With 3.6 g PSE/d	Difference	
Total carotenoids <sup>b</sup>	116 ± 2.7	105 ± 2.7	-11.2 ± 3.5	0.0022
β-Carotene	21 ± 0.7	18 ± 0.7	-2.7 ± 0.75	0.0009
α-Carotene	11 ± 0.4	9 ± 0.4	-1.4 ± 0.57	0.0226
Lutein + zeaxanthin	15 ± 0.4	15 ± 0.4	0 ± 0.4	0.9980
Anhydrolutein	11 ± 0.3	11 ± 0.3	0 ± 0.4	0.9054
α-Cryptoxanthin	7 ± 0.2	6 ± 0.3	-0.5 ± 0.3	0.0691
β-Cryptoxanthin, males	10 ± 0.5	10 ± 0.5	-0.2 ± 0.48	0.6509
β-Cryptoxanthin, females	11 ± 0.6	10 ± 0.6	-1.5 ± 0.65	0.0312
Phytofluene	6 ± 0.2	5 ± 0.2	-0.5 ± 0.19	0.0110
α-Tocopherol	1360 ± 55	1276 ± 55	-85 ± 46	0.0669
γ-Tocopherol	205 ± 11	204 ± 11	-1.4 ± 11	0.8947
Retinol	41 ± 2	39 ± 2	-2 ± 1.8	0.3358

<sup>a</sup>Estimated mean and standard error of the estimate from a mixed model analysis of variance that adjusted for baseline subject characteristics, type of dressing (ranch or Italian), period, and carryover and covariance with blood lipids and lipoproteins; *n* = 53 (26 men, 27 women).

<sup>b</sup>There was a significant interaction of treatment (PSE) with body mass index (BMI) for lycopene; thus, LSMeans are uninformative. The decrease in lycopene due to PSE consumption was significant at BMI = 23 (-4.93 ± 2.01 µg/dL or -13.2%; *P* = 0.0181). At BMI = 30, lycopene decreased by 10.9 ± 2.10 µg/dL or 29% (*P* < 0.0001). For abbreviation see Table 2.

blood lipid or lipoprotein was independent of the baseline concentration. Because baseline was a significant indicator of the final concentration, it was included in all models. The importance of the relationship of baseline LDL cholesterol concentration to LDL cholesterol in both control and PSE diets is depicted graphically in Figure 1 for men and women.

There were no significant effects on blood lipids and lipoproteins due to gender, age, or BMI. In addition, there were no significant interactions of gender, age, or BMI with dressing or treatment, indicating that responses to dressing and PSE were also independent of these subject characteristics. Note that these relationships of blood lipids and PSE to

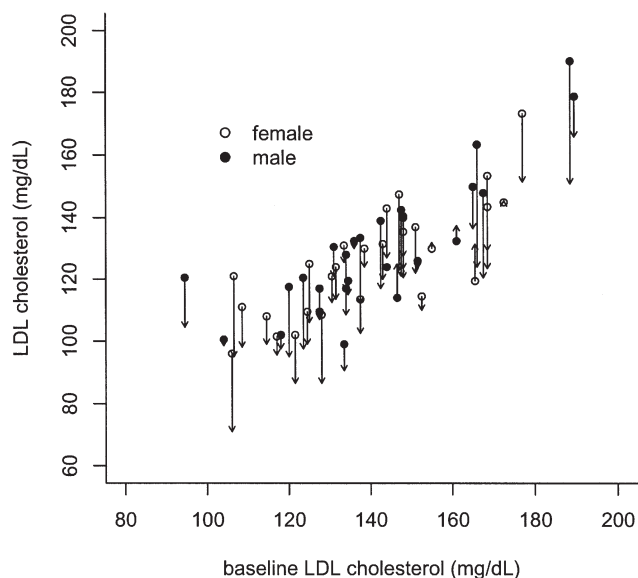
**TABLE 5**  
**Effect of Subject Characteristics Prior to Dietary Intervention on Plasma Lipid and Lipoprotein Changes due to Consumption of Plant Sterol Esters (PSE)**

Independent variables <sup>b</sup>	Dependent variable (Y) estimated by the model <sup>a</sup>					
	Total cholesterol (TC)	Triglycerides (TG)	LDL cholesterol	Apolipoprotein B	HDL cholesterol	Apolipoprotein A1
	Mean of the coefficient for independent variable ± SEE ( <i>P</i> -value)					
Intercept	78 ± 12 (NS) <sup>c</sup>	20 ± 9 (0.0224)	11 ± 8 (NS)	42 ± 46 (NS)	6 ± 2 (0.0048)	366 ± 104 (0.0011)
Baseline concentration of Y	0.82 ± 0.05 (<0.0001)	0.61 ± 0.06 (<0.0001)	0.74 ± 0.06 (<0.0001)	8.9 ± 0.5 (<0.0001)	0.85 ± 0.04 (<0.0001)	7.8 ± 0.5 (<0.0001)
Dressing	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)
Treatment	-13.8 ± 1.62 (<0.0001)	-8.50 ± 2.88 (0.0047)	-12.5 ± 1.48 (<0.0001)	-30.4 ± 7.1 (<0.0001)	-0.42 ± 0.44 (NS)	-9.49 ± 16.2 (NS)
Period	(NS)	(NS)	(NS)	(NS)	(NS)	68.7 ± 21.5 (0.0024)
Interaction among independent variables						
Dressing with period	5.36 ± 2.16 (0.0162)	(NS)	(NS)	30.6 ± 9.4 (0.0021)	1.57 ± 0.60 (0.0121)	(NS)
Gender (male) with age	(NS)	0.40 ± 0.14 (0.0064)	(NS)	(NS)	(NS)	(NS)
Gender with period	(NS)	(NS)	(NS)	(NS)	(NS)	-64.7 ± 28.8 (0.0292)

<sup>a</sup>Dependent variable (Y) estimated by:  $Y$  (mg/dL) = Intercept +  $b$  +  $b^2$  + Dressing + Treatment + Diet Sequence + Period + Age + BMI + TC + TG + (TC + TG) + LDL cholesterol + HDL cholesterol + interactions among independent variables, where  $b$  = baseline concentration of dependent variable Y; Dressing = ranch vs. Italian. BMI, body mass index; for other abbreviations, see Table 1.

<sup>b</sup>There were no significant main effects of sequence of the diet administration, gender, age, or BMI of the participants (data omitted from the table).

<sup>c</sup>NS, not significant.



**FIG. 1.** Preintervention (baseline) plasma low density lipoprotein (LDL) cholesterol concentration vs. change in LDL cholesterol in response to diets supplemented with 3.6 g/d of plant sterol esters (PSE) prepared from vegetable oil. For each participant, an arrow indicates the direction and magnitude of change from the control diet ( $-PSE$ ) to the intervention diet ( $+PSE$ ). Baseline concentrations for men and women were distributed similarly, as were responses to the intervention diet.

age and BMI cannot be extrapolated to a wider range than that in this study because age and BMI were selection criteria.

There was no effect of controlled diets with or without PSE on Lp(a) (data not shown).

*Relationships of baseline subject characteristics with plasma carotenoid responses to PSE, after adjusting for blood lipid changes.* In modeling responses of carotenoids to PSE, we were interested in determining if blood lipid concentrations were a determinant of carotenoid response. In addition to the independent and design variables considered for blood lipids and lipoproteins, TG, TC, LDL cholesterol, HDL cholesterol, and total plasma lipids (estimated as the sum of TG + TC) were included as candidate covariates in modeling carotenoid responses. Baseline carotenoid concentration was a significant predictor of final carotenoid concentration for all carotenoids (Table 6). As observed for blood lipids, carotenoid responses were independent of dressing at the  $P = 0.05$  level of significance. There were no significant interactions of baseline plasma carotenoid concentration with effects of dressing or treatment for any carotenoid.

After adjusting for the blood lipid covariates, baseline concentration of the dependent carotenoid variable, subject characteristics, and variables associated with study design (Table 6), there remained significant effects of PSE for total carotenoids,  $\beta$ -carotene, and  $\alpha$ -carotene. Thus, blood lipid responses to the diets do not completely determine the plasma carotenoid responses to PSE consumption.

## DISCUSSION

In this study, a large dietary trial showed that reduced-fat and low-fat salad dressings are effective carriers of PSE prepared from soybean oil. The dressings were compared within diets that were balanced for fat, fatty acids, and other nutrients. The reduction in CVD risk factors was not affected by the amount of fat in the dressing that carried the PSE. When two servings per day were fed as part of a mixed diet, the reduced-fat ranch salad dressing (8 g of fat per serving) and the low-fat Italian dressing (4 g of fat per serving), each supplemented with 1.1 g of free sterol equivalent per serving, resulted in approximately equal reductions in the major blood lipids. Each dressing was compared to a control dressing of similar fat and fatty acid composition but with no added PSE.

The effectiveness of reduced- and low-fat foods as carriers of PSE was demonstrated by highly significant reductions in blood lipid risk factors for CVD. TC and LDL cholesterol, indicators of increased risk, declined about 7% and 10%, respectively, with consumption of 3.6 g PSE/d, while HDL cholesterol, associated with reduced risk (19,20), remained unchanged. Lower ratios of TC to HDL cholesterol have been postulated to be strong, independent indicators of decreased risk for CVD (21,22). As would be expected considering the observation of major reductions in LDL cholesterol and sparing of HDL cholesterol by PSE consumption in the current study, the ratio of TC to HDL cholesterol was reduced by 6.9–7.7%, again indicating decreased risk for CVD with consumption of PSE. Increased blood TG concentrations have also been postulated as a risk factor for CVD (23). TG concentrations were reduced by 7% by PSE.

Changes in the major apolipoproteins of LDL (Apo B) and HDL (Apo A1) and changes in cholesterol associated with the corresponding lipoprotein were directionally similar. Similar changes in blood cholesterol and apolipoproteins have been shown in other studies of PSE (4,5). We did not directly determine lipoprotein particle composition. However, changes in LDL cholesterol and Apo B were directionally similar but not directly proportional. This may indicate a reduction in the cholesterol content of the lipoprotein particle as well as a reduction in the number of particles. Lp(a), similar to LDL in lipid composition and with Apo B as one major apolipoprotein but with a second major apolipoprotein referred to as Apo (a), may be a risk factor for the development of CVD (24,25). The particle has been reported to be more atherogenic than LDL (26,27). In our studies, and in those of others, Lp(a) has been altered by changes in dietary fatty acid intake. SFA tend to lower Lp(a) (28–30), whereas *trans* monounsaturated fatty acids raise Lp(a) (31,32). Although we did not expect that PSE would affect Lp(a) because the reputed action site of PSE lies ex-systemically at the level of the gastrointestinal tract (33), nonetheless it is important to know whether this highly atherogenic particle is affected by PSE in the diet before we can evaluate the overall reduction in CVD risk associated with PSE consumption. In the current study, Lp(a) was unaffected by inclusion of PSE in the diet.

TABLE 6

Effect of Subject Characteristics Prior to Dietary Intervention on Plasma Carotenoid Changes due to Consumption of Plant Sterol Esters (PSE)

Independent variables <sup>b</sup>	Dependent variable (Y) estimated by the model <sup>a</sup>					
	Total carotenoids	$\beta$ -Carotene	$\alpha$ -Carotene	Lycopene	Lutein + zeaxanthin	Anhydrolutein
	Mean of the coefficient for independent variable and SEE (P-value)					
Intercept	3.8 $\pm$ 13 (NS)	12 $\pm$ 2 ( $<0.0001$ )	5 $\pm$ 0.6 ( $<0.0001$ )	18 $\pm$ 3 ( $<0.0001$ )	-1.4 $\pm$ 2.4 (NS)	-4 $\pm$ 2 (0.0446)
Baseline concentration of Y	0.48 $\pm$ 0.06 ( $<0.0001$ )	0.33 $\pm$ 0.08 ( $<0.0001$ )	0.42 $\pm$ 0.04 ( $<0.0001$ )	0.28 $\pm$ 0.04 ( $<0.0001$ )	0.49 $\pm$ 0.05 ( $<0.0001$ )	0.48 $\pm$ 0.06 ( $<0.0001$ )
Dressing	(NS) <sup>c</sup>	(NS)	(NS)	(NS)	(NS)	(NS)
Treatment <sup>d</sup>	-11.2 $\pm$ 3.48 (0.0022)	-2.65 $\pm$ 0.75 (0.0009)	-1.35 $\pm$ 0.57 (0.0226)	-14.7 $\pm$ 10.9 (NS)	-0.0 $\pm$ 0.41 (NS)	-0.05 $\pm$ 0.38 (NS)
Sequence <sup>e</sup>	(NS)	-7.71 $\pm$ 2.38 (0.0022)	(NS)	(NS)	(NS)	(NS)
Period	(NS)	(NS)	(NS)	-4.45 $\pm$ 1.47 (0.0039)	(NS)	(NS)
Total cholesterol (TC) <sup>f</sup>	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)
Triglycerides (TG) <sup>f</sup>	(NS)	(NS)	(NS)	0.07 $\pm$ 0.02 (0.0006)	(NS)	(NS)
TC + TG <sup>f</sup>	(NS)	(NS)	(NS)	(NS)	0.02 $\pm$ 0.01 (0.0009)	0.02 $\pm$ 0.01 ( $<0.0001$ )
LDL cholesterol <sup>f</sup>	0.32 $\pm$ 0.11 (0.0055)	(NS)	(NS)	(NS)	(NS)	(NS)
HDL cholesterol <sup>f</sup>	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)
Interaction of treatment with other variables						
Treatment with BMI	(NS)	(NS)	(NS)	-0.85 $\pm$ 0.41 (0.0427)	(NS)	(NS)

<sup>a</sup>Dependent variable (Y) estimated by:  $Y$  (mg/dL) = Intercept +  $b$  +  $b^2$  + Dressing + Treatment + Diet Sequence + Period + Age + BMI + TC + TG + (TC + TG) + LDL cholesterol + HDL cholesterol + Interactions among independent variables, where  $b$  = baseline concentration of dependent variable Y; Dressing = ranch vs. Italian. BMI, body mass index; for other abbreviations see Table 1.

<sup>b</sup>There were no significant main effects of gender, age, or BMI of the participants (data omitted from the table).

<sup>c</sup>NS, not significant.

<sup>d</sup>Treatment = +PSE vs. -PSE.

<sup>e</sup>Diet sequences: Baseline to -PSE to +PSE vs. Baseline to +PSE to -PSE.

<sup>f</sup>Covariates: TC, TG, LDL cholesterol, HDL cholesterol, and sum of TC and TG.

In the 1994 report of strategies for blood cholesterol reduction from the National Cholesterol Education Program (NCEP) (34), it was estimated that for every 1% reduction in cholesterol concentration, the risk of CVD decreased by an average of 2%. Application of this prediction to TC reduction observed in the present study would indicate an average reduction in risk for CVD of 28%. This is based on changes from initial (baseline) plasma TC concentrations when a moderately low-fat, low-SFA diet with 3.6 g PSE/d was consumed. This may be partitioned as 14% reduction in risk due to consumption of the reduced-SFA diet without PSE, with a further reduction of 14% due to addition of PSE to the diet. Thus PSE consumption results in a reduction in risk beyond that resulting from reduced consumption of SFA. From this, we postulate that inclusion of PSE in the diet may have benefits unrelated to saturated fat intake and that even those people unwilling to commit to changes in type or amount of fat intake may benefit significantly from consumption of food products containing PSE.

When effects of PSE consumption (and type of dressing) were modeled, baseline (preintervention) concentration of a

plasma constituent was always an important predictor of final concentration. However, there was no interaction of baseline concentrations with response to PSE for any plasma lipid or lipoprotein. This indicates that people may benefit from a reduction in CVD risk with consumption of PSE regardless of their starting blood cholesterol level. For individuals having a normal cholesterol level, regular PSE consumption may help prevent the development of elevated cholesterol often associated with aging and changes in lifestyle as we age.

Unlike plasma lipids and lipoproteins, decreases in the TC/HDL cholesterol ratio with PSE consumption was related to the ratio at entrance of the subjects into the dietary intervention. Thus, if the ratio is an independent indicator of CVD risk (19,20), people with higher ratios, and thus at higher risk, may benefit from a greater reduction in risk when they consume PSE than do those who have lower ratios.

In this investigation, we found that plasma carotenoid concentrations decreased with addition of PSE to the diet. For example,  $\beta$ - and  $\alpha$ -carotene both decreased by about 13%, while lycopene decreased by about 20%. Plasma concentrations of fat-soluble nutrients such as carotenoids may be lowered by

PSE either directly by reduced absorption or indirectly through undefined mechanisms. Plasma concentrations of some, if not all, carotenoids may be related to the concentration of the plasma lipoproteins on which they are carried (16) and may decrease or increase as plasma lipoproteins change. Thus, in experimental studies, fat-soluble carotenoids and vitamins are commonly normalized to plasma lipid concentrations. This may not be appropriate, however, when both the carotenoid and the plasma lipid are dependent variables and both are subject to change with treatment.

We saw no evidence that PSE consumption affected fat-soluble vitamin absorption. However, this investigation was conducted over a relatively short time period, and results cannot be extrapolated to what might occur over longer periods. Concentrations of plasma carotenoids in this study remained well within what may be considered normal levels. Again, as for the fat-soluble vitamins, caution must be exercised in extrapolating our results to long-term effects on carotenoids. However, plasma carotenoids are strongly related to dietary intake. On diets sufficiently rich in carotenoids, we expect that plasma concentrations of carotenoids will reach a lower plateau due to PSE consumption but will remain within normal ranges. The effects of PSE on plasma carotenoid concentrations when the diet is marginal or low in carotenoids may be of more concern.

This study did not directly compare salad dressings with vegetable oil spreads, which are the common form of commercially available food carriers of PSE. However, the extent of LDL cholesterol lowering by PSE delivered by dressings in this study, averaging 17% compared with the subjects' cholesterol level at entry, and 9.7% compared with the control diet, matched what can be extrapolated from clinical studies in which PSE were delivered by spreads having a higher fat content (4,5). In theory, there should not be any difference in the *in vivo* digestion of the fat in a spread product and the fat in a dressing product despite the differences in how they are structured. Each type of product should deliver PSE to the digestive milieu. Our data clearly support this supposition.

Reduction in SFA intake remains the primary dietary goal for individuals and for the population at large to lower LDL cholesterol and reduce the risk of CVD. In the 2001 report from the NCEP on the treatment of high blood cholesterol in adults, Adult Treatment Panel III (ATP-III) (35), the recommended intake of saturated fat is less than 7% of energy with total fat intake of 25–35% of energy. Food carriers of PSE including both vegetable oil-based spreads and reduced-fat salad dressings, each having high levels of polyunsaturated fatty acids, can be incorporated in NCEP ATP-III type diets. However, dressings with lower total fat/serving may be more readily used to deliver PSE while replacing SFA. For example, two servings per day of low-fat salad dressing, such as the Italian dressing fed in this study, carrying 3.6 g of PSE, would provide about 8 g of fat and 1 g of SFA per day but would deliver PSE in amounts that significantly reduce blood cholesterol risk of CVD. For women consuming 2163 kcal per day, the average intake in this study, two servings per day of the Italian dressing added to a

diet having 30% of calories from fat would provide only 11% of the daily fat intake and 5% of the SFA recommended as the maximum SFA intake in an NCEP ATP-III type diet. Corresponding amounts for men consuming 2766 kcal per day (the average energy intake in this study) would be 9% of daily fat intake and 4% of the SFA intake. These amounts of fat are readily balanced by removal of dietary fat from other sources and would be especially beneficial if the fat replaced is animal fat or vegetable oil-containing products high in either SFA or *trans* unsaturated fatty acids.

We conclude that low-fat foods, such as reduced- and low-fat salad dressings, are effective carriers for PSE. Such reduced-fat foods can be readily included in diets low in SFA as well as diets moderately low in total fat, i.e., prudent diets, to lower blood cholesterol and reduce the risk of CVD. Furthermore, the reductions in LDL cholesterol and in the ratio of TC to HDL cholesterol are not conditioned by the plasma lipid level of the person when consumption begins. Thus, PSE addition to the diet, coupled with the primary goal of reducing saturated fat intake, may help to both prevent development of high LDL cholesterol and reduce CVD risk.

## ACKNOWLEDGMENT

Partial financial support was provided by Unilever Bestfoods NA (formerly Lipton) through a Research Support Agreement with the Agricultural Research Service, USDA.

## REFERENCES

1. Dietary Guidelines Advisory Committee (2000) Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans, 2000, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD.
2. Pollak, O.J., and Kritchevsky, D. (1981) *Sitosterol (Monographs on Atherosclerosis, Vol. 10)*, Karger, Basel.
3. de Vries, J.H.M., Kromhout, J.A., van de Bovenkamp, P., van Straveren, W.A., Mensink, R.P., and Katan, M.B. (1997) The Fatty Acid and Sterol Content of Food Composites of Middle-Aged Men in Seven Countries, *J. Food Comp. Anal.* 10, 115–141.
4. Weststrate, J.A., and Meijer, G.W. (1998) Plant Sterol-Enriched Margarines and Reduction of Plasma Total- and LDL-Cholesterol Concentrations in Normocholesterolaemic and Mildly Hypercholesterolaemic Subjects, *Eur. J. Clin. Nutr.* 52, 334–343.
5. Hendriks, H.F.J., Weststrate, J.A., van Vliet, T., and Meijer, G.W. (1999) Spreads Enriched with Three Different Levels of Vegetable Oil Sterols and the Degree of Cholesterol Lowering in Normocholesterolaemic and Mildly Hypercholesterolaemic Subjects, *Eur. J. Clin. Nutr.* 53, 319–327.
6. Society of Actuaries and Association of Life Insurance Medical Directors of America 1979 (1980) *Weight of Insured Persons in the United States Associated with Lowest Mortality*, Association of Life Insurance Medical Directors of America, Philadelphia.
7. U.S. Department of Agriculture, Agricultural Research Service (1998) USDA-NCC Carotenoid Database for U.S. Foods—1998, Nutrient Data Laboratory Home Page, <http://www.nal.usda.gov/fnic/foodcomp>.
8. National Heart Lung and Blood Institute (1982) Lipid and Lipoprotein Analysis, in *Manual of Laboratory Operations, Lipid Research Clinics Program* (Hainline, A., Karon, J., and Lippel, K., eds.), 2nd edn., Department of Health and Human Services, Bethesda, MD.

9. Gidez, L.I., Miller, G.J., Burstein, M., Slagle, S., and Eder, H.A. (1982) Separation and Quantitation of Subclasses of Human Plasma High-Density Lipoproteins by a Simple Precipitation Procedure, *J. Lipid Res.* 23, 1206–1223.
10. Friedewald, W.T., Levy, R.I., and Fredrickson, D.S. (1972) Estimation of the Concentration of Low-Density Lipoprotein Cholesterol Without Use of the Preparative Ultracentrifuge, *Clin. Chem.* 18, 499–502.
11. Khachik, F., Beecher, G.R., Goli, M.B., Lusby, W.R., and Smith, J.C. (1992) Separation and Identification of Carotenoids and Their Oxidation Products in the Extracts of Human Plasma, *Anal. Chem.* 64, 2111–2122.
12. Bieri, J.G., Brown, E.D., and Smith, J.C. (1985) Determination of Individual Carotenoids in Human Plasma by High Performance Liquid Chromatography, *J. Liq. Chromatogr.* 8, 473–484.
13. Burnham, K.P., and Anderson, D.R. (1998) *Model Selection and Inference; A Practical Information-Theoretic Approach*, Springer-Verlag, New York.
14. Littell, R.C., Milliken, G.A., Stroup, W.W., and Wolfinger, R.D. (1996) *SAS System for Mixed Models*, SAS Institute, Inc., Cary, NC.
15. Schwartz, G. (1978) Estimating the Dimension of a Model, *Ann. Statistics* 6, 461–464.
16. Clevidence, B.A., and Bieri, J.G. (1993) Association of Carotenoids with Human Plasma Lipoproteins, *Methods Enzymol.* 214.
17. U.S. Department of Agriculture, Agricultural Research Service, (1996) Data Tables: Results for USDA's 1995 Continuing Survey of Food Intakes by Individuals and 1995 Diet and Health Knowledge Survey, Beltsville Human Nutrition Research Center, Food Surveys Research Group, Beltsville, MD.
18. U.S. Department of Agriculture, Human Nutrition Information Service (1976–1990) Composition of Foods, *Agriculture Handbook No. 8*, Sections 1–22, U.S. Government Printing Office, Washington, DC.
19. Miller, G.J., and Miller, N.E. (1975) Plasma High-Density Lipoprotein Concentration and Development of Ischemic Heart Disease, *Lancet* i, 16–20.
20. Gordon, D.J., Probstfield, J.L., Garrison, R.J., Neaton, J.D., Castelli, W.P., Knoke, J.D., Jacobs, D.R., Jr., Bangdiwala, S., and Tyroler, H.A. (1989) High-Density Lipoprotein Cholesterol and Cardiovascular Disease: Four Prospective American Studies, *Circulation* 79, 8–15.
21. Lichtenstein, A.H., Ausman, L.M., McNamara, J.R., and Schaefer, E.J. (1996) *Trans* and Saturated Fatty Acid Content of Dietary Fat Effects Plasma Lipid and Lipoprotein Concentrations, *Circulation* 94, 1–97.
22. Chisholm, A., Mann, J., Sutherland, W., Duncan, A., Skeaff, M., and Frampton, C. (1996) Effect on Lipoprotein Profile of Replacing Butter with Margarine in a Low Fat Diet: Randomized Crossover Study with Hypercholesterolaemic Subjects, *Br. Med. J.* 312, 931–934.
23. Hokanson, J.E., and Austin, M.A. (1996) Plasma Triglyceride Level Is a Risk Factor for Cardiovascular Disease Independent of High-Density Lipoprotein Cholesterol Level: A Meta-analysis of Population-Based Prospective Studies, *J. Cardiovasc. Risk* 3, 213–229.
24. Bostom, A.G., Gagnon, D.R., Cupples, L.A., Wilson, P.W.F., Jenner, J.L., Ordovas, J.M., Schaefer, E.J., and Castelli, W.P. (1994) A Prospective Investigation of Elevated Lipoprotein(a) Detected by Electrophoresis and Cardiovascular Disease in Women: The Framingham Heart Study, *Circulation* 90, 1688–1695.
25. Schaefer, E.J., Lamon-Fava, S., Jenner, J.L., McNamara, J.R., Ordovas, J.M., Davis, E., Abolafia, J.M., Lippel, K., and Levy, R.I. (1994) Lipoprotein (a) Levels and Risk of Coronary Heart Disease in Men: The Lipid Research Clinics Coronary Primary Prevention Trial, *J. Am. Med. Assoc.* 271, 999–1003.
26. Hoefler, G., Harnoncourt, F., Paschke, E., Mirtl, W., Pfeiffer, K.H., and Kostner, G.M. (1988) Lipoprotein Lp(a): A Risk Factor for Myocardial Infarction, *Arteriosclerosis* 8, 398–401.
27. Rosengren, A., Wilhelmsen, L., Eriksson, E., Risberg, B., and Wedel, H. (1990) Lipoprotein (a) and Coronary Heart Disease: A Prospective Case-Control Study in a General Population Sample of Middle Aged Men, *Br. Med. J.* 301, 1248–1251.
28. Clevidence, B.A., Judd, J.T., Schaefer, E.J., Jenner, J.L., Lichtenstein, A.H., Muesing, R.A., Wittes, J.A., and Sunkin, M.E. (1997) Plasma Lipoprotein(a) Levels in Men and Women Consuming Diets Enriched in Saturated, *cis* or *trans* Monounsaturated Fatty Acids, *Arterioscler. Thromb. Vasc. Biol.* 17, 1–5.
29. Almendingen, K., Jordal, O., Kierulf, P., Sandstad, B., and Pedersen, J.I. (1995) Effects of Partially Hydrogenated Fish Oil, Partially Hydrogenated Soybean Oil, and Butter on Serum Lipoproteins and Lp(a) in Men, *J. Lipid Res.* 36, 1370–1384.
30. Judd, J.T., Baer, D.J., Clevidence, B.A., Muesing, R.A., Chen, S.C., Weststrate, J.A., Meijer, G.W., Wittes, J., Lichtenstein, A.H., Vilella-Bach, M., and Schaefer, E.J. (1998) Effects of Margarine Compared with Those of Butter on Blood Lipid Profiles Related to Cardiovascular Disease Risk Factors in Normolipemic Adults Fed Controlled Diets, *Am. J. Clin. Nutr.* 68, 768–777.
31. Mensink, R.P., Zock, P.L., Katan, M.B., and Hornstra, G. (1992) Effect of Dietary *cis* and *trans* Fatty Acids on Serum Lipoprotein(a) Levels in Humans, *J. Lipid Res.* 33, 1493–1501.
32. Nestel, P., Noakes, M., Belling, B., McArthur, R., Clifton, P., Janus, E., and Abbey, M. (1992) Plasma Lipoprotein Lipid and Lp(a) Changes with Substitution of Elaidic Acid for Oleic Acid in the Diet, *J. Lipid Res.* 33, 1029–1036.
33. Normen, L., Dutta, P., Lia, A., and Andersson, H. (2000) Soy Sterol Esters and  $\beta$ -Sitosterol Ester as Inhibitors of Cholesterol Absorption in Human Small Bowel, *Am. J. Clin. Nutr.* 71, 908–913.
34. National Cholesterol Education Program (1994) Second Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II), *Circulation* 89, 1329–1445.
35. National Cholesterol Education Program (2001) Executive Summary: Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), *J. Am. Med. Assoc.* 285, 2486–2497.

[Received April 27, 2001, and in revised form July 27, 2001; revision accepted October 2, 2001]